(12) UK Patent Application (19) GB (11) 2 212 810 (13) A

(43) Date of A publication 02.08.1989

(21) Application No 8824694.7

(22) Date of filing 21.10.1988

(30) Priority data
(31) 4723 (32) 21.10.1987 (33) HU

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(51) INT CL⁴
C08G 69/08 // A01N 37/18 , A61K 31/785 37/02

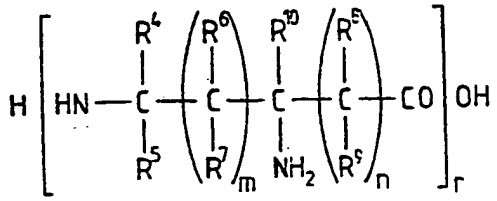
(52) UK CL (Edition J)
C3R RPA RPB RPC RPX RSM RSP RSX R22D3A R22D3B R22MX R22N1A R22N1B R22PX R22P1 R22T2
A5B BHA B180 B31Y B317
A5E EAB E202 E237 E239 E247 E274 E279
U1S S1304 S1313 S2410

(56) Documents cited
Indian J Chem, 21B, 483, (1982)
Indian J Exper. Biology, 20,227 (1982)
Int. J Peptide Prot. Res, 17, 189, (1981)
Biopolymers, 19, 219 (1980)
Int. J Biol. Macromol, 2, 53, (1980)
Biopolymers, 17, 2427, (1978)

(58) Field of search
UK CL (Edition J) C3R RA RPA RPB RPC RPX RSM RSP RSX
INT CL⁴ C08G

(54) Isopolypeptides and process for the preparation thereof

(57) The invention relates to isopolypeptides of the general formula (I)



(I)

as well as their salts, racemates and optical isomers, and to a process for preparing these compounds.

The compounds exert an antitumour and antiviral effect.

In the above formula R^4 to R^{10} are each hydrogen or C_{1-4}–alkyl, r is 10 to 400, m is 0 to 10 and n is 0 to 10. Compounds of the above formula wherein m is 0 to 3, n is 0 and R^8 and R^9 are each hydrogen are defined per se.

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α-polylysine (A-3) caused only stagnation of cell number.

According to our in vivo experiments SZTP treatment resulted in the increased survival of the treated animals. In case of Ehrlich tumour the treatment caused a very long tumour-free period and tumour growth was observed only after the cessation of treatment.

SZTP treatment completely prevented metastasis formation in the liver when the tumour was inoculated into the spleen, and decreased the number and size of lung metastases when the tumour was inoculated into the muscle.

Accordingly, the polyisolysines and polyisoornithines produced according to the invention proved to be inhibitors of tumour-cell proliferation. Among the compounds poly-iso--lysines showed a surprisingly strong inhibitory effect. This effect can be readily compared to the effect of the known best cytostatic drugs based upon the survival of the animals. The unexpectedly good antimetastatic effect of our compounds is evident. An extra benefit of our compounds is their low toxicity which is far less than that of the cytostatic drugs applied till now.

Our biological investigations showed for instance that the therapeutic index $\frac{\text{maximal tolerable dose}}{\text{minimal effective dose}}$ is a value over 10, while this value is usually well below 10 in case of most of the known cytostatic drugs.

No difference was observed between the biological response of males and females in this respect, which is also a benefit.

Table 1b shows, however, that in contrary, H-17 and K-17 did not influence the proliferation of K-562 cells.

Table 1c shows that α-polylysine caused the stagnation of cell number when compared to the control but it did not cause increase of cell number even in high dose (100 µg/ml).

The values shown in the Tables mean the cell number in the given points in time. Cells were counted in 3 test tubes pro dose and in 3 test tubes as control in each point of time.

Table 1a

The effect of compounds prepared according to the invention (SZTP-14-15-16-17-18) on the K-562 cell line, in vitro.

Treatment: 1-10-100 µg/ml, 24 hours after the dilution of culture

filling and additive agents are used as carriers, e.g. milk sugar, magnesium stearate, saccharose, talc, stearic acic, gelatine, agar-agar, pectin, tragacant, collodial silica gel, corn starch, algic acid etc. For making suppositories, advantageously cacao butter is used.

The treatment of plants is expediently carried out by transforming the compounds according to the invention with usual auxiliary agents such as moistening materials and solid carriers, preferably caolin, to solid or liquid preparations, preferably in combination with other active substances. From the thus-obtained preparation a solution or suspension containing the compound according to the invention in a concentration of 1 to 50 ppm is made and it is applied in a known way to the plants, preferably in an amount of 0.5 to 5 g/ha.

The invention is further illustrated by the following non-limiting Examples.

Example 1

Poly-L-lysine HBr salt

a) N^{α}-Benzyloxycarbonyl-L-lysine-p-nitrophenylester.HCl

10 g of N^{α}-benzyloxycarbonyl-(N^{ε}-tert.butyloxycarbonyl)-L-lysine-p-nitrophenyl ester was dissolved in 40 ml of trifluoroacetic acid and an equivalent amount of 2N HCl in EtOAC was added. After standing for one hour at room temperature the solution was evaporated in vacuo to half volume, then ether was added and the solution was crystallized. Yield: 8.3 g (96.06%); m.p. 83-86 °C; TLC R_f = 0.68

(n-butanol-acetic acid-water 4 : 1 : 1).

b) N^{α}-Benzyloxycarbonyl-N-(N'^{α}-benzyloxycarbonyl-N'^{ε}-tert.butyloxycarbonyl- L-lysil)-L-lysine-p--nitrophenylester

7.69 g (20 mmole) of N^{α}-benzyloxycarbonyl-(N^{ε}-tert.butyloxycarbonyl)-L-lysine were dissolved in abs. acetonitrile. 2.8 ml (20 mmole) of triethylamine and then 2.8 ml of isobutylchloroformate were added dropwise, under vigorous stirring. After activation (20 min.) 9 g of the salt of step a) were added, followed by dropwise addition of a cold solution of 2.8 ml of triethylamine in 5 ml of acetonitrile. Stirring was continued for 2 hours at -10 °C. The mixture was diluted with 300 ml of 0.5 M HCl. The solid product was collected, dried and recrystallized from a 1 : 1 mixture of ethyl acetate and petroleum ether. Yield: 12.9 g (89.02%); m.p.: 126-131 °C. Analysis: -ONP content 59.8%. TLC R_f = 0.64 (EtOAc-cyclohexane 3 : 1). R_f = 0.91 (n-butanol--acetic acid-water 4 : 1 : 1); HPLC tr. = 8.8 min. (k' = 2.2); column: ODS-Hypersil-6 (125x4 mm); eluent: MeOH-CH_3CN-0.02 M NaOAc buffer (pH4) 40 : 30 : 30 v/v; flow rate: 1 ml/min.; detection: UV 254 nm.

c) N^{α}-Benzyloxycarbonyl-N^{ε}-(N'^{α}-benzyloxycarbonyl-L--lysine)-L-lysine-p-nitrophenylester-hydrochloride

From 10.5 g of N^{α}-benzyloxycarbonyl-N-(N'^{α}-benzyloxycarbonyl-N'^{ε}-tert.butyloxycarbonyl-L-lysil)-L-lysine-p--nitrophenylester 10.25 g of salt were produced according to step a). M.p.: 109-113 °C. TLC R_f = 0.70 (n-BuOH-AcOH--H_2O 4 : 1 : 1).

c) N^{ε}-tert.-Butyloxycarbonyl-tri(N^{α}-benzyloxycarbonyl-L-lysine)-p-nitrophenylester

5.71 g (15 mmole) of N^{α}-benzyloxycarbonyl-(N^{ε}-tert.-butyloxycarbonyl)-L-lysine were transformed into mixed anhydride, as in step b), with triethylamine, isobutylchloroformate in acetonitrile. 10.5 g of N^{α}-benzyloxycarbonyl-N^{ε}(N'^{α}-benzyloxycarbonyl-L-lysil)-L-lysine-p-nitrophenylester hydrochloride were added in the presence of triethylamine. By working up as in step b) 13.9 g product were obtained (85.4%). It was recrystallized from acetonitrile and ether. M.p.: 145-150 °C. Analysis: ONP content 99.6%; TLC R_f = 0.98 (n BuOH-AcOH-H_2O 4 : 1 : 1); HPLC tr. = 18.2 min. (k' = 8.1); column: ODS-Hypersil-6 (125x4 mm); eluent: MeOH-CH_2CN-0.02 M NaOAc buffer (pH4) 40 : 30 : 30); flow rate: 1 ml/min.; detection: UV 254 nm.

e) Tri-(N^{α}-benzyloxycarbonyl-L-lysine)-p-nitrophenylester-hydrochloride

12.2 g of ester prepared in step d) were treated as in step a). In this way 12.1 g (95.4%) of the salt were obtained; m.p.: 108-125 °C. TLC R_f = 0.71 (n-BuOH:AcOH:H_2O 4 : 1 : 1).

f) Poly-ε-N^{α}-benzyloxycarbonyl-L-lysine

23 g (24 mmole) of tri-(N^{α}-benzyloxycarbonyl-L-lysine)-p-nitrophenylester-hydrochloride were condensed in 60 ml of dimethylsulfoxide, in the presence of 4.5 ml of triethylamine, up to gel formation. The polymer was isolated by pouring into an aqueous 5% Na_2CO_3 solution. Yield: 16.8 g (89.6%). Analysis: Z_{CO_2} : 16.8% (calc.).

16.3% (found); $[\eta_{sp}]/c$ = 31.92 cm^3/g (c = ~.95 g %, dichloroacetic acid).

g) Poly-ε-L-lysine hydrobromide

16 g of protected polymer of step f) were dissolved in 100 ml of trifluoroacetic acid and 100 ml of 4N HBr/AcOH were added. The end-product was precipitated by ether. Yield: 14.7 g (91.6%). Analysis: Br: 37.7% (calc.), 36.8% (found); $A_{254\ nm}$ = 0. Paper chromatography: Wl 23x62, eluent: n-BuOH-AcOH-Pyr.-H_2O 30:6:24:20. Yield summarized: 36.82% (starting from protected monomer); 18.85% (from free lysine). $M\bar{W}$ = 5500 - 20,300 D.

h) Purification of the crude end-product

250 g of the polymer obtained in step g) were dissolved in water and then chromatographed on Sephadex G-50 (fine) column. Eluent: dist. water, 0.9% NaCl solution or 0.1 N HCl solution. Detection for fractions (1 ml) at UV 220 nm.

i) Purification of the crude end-product

2 g of poly-ε-L-lysine hydrobromide prepared in step g) were dissolved in 2 ml of water, then 20 ml of ethanol were added. The polymer was precipitated by 80 ml of diethyl ether. After 24 hours of standing at 5 °C it was filtered, washed and dried. Yield: 1.26 g (83%) (57.7% when calculated for the starting protected tripeptide).

The thus-obtained substance is designated as SZTF-14; $M\bar{W}$ = 12,700 D; $[\alpha]_D^{20}$ = +32.4° (c = 2; H_2O); polydispersity = 3.7.

The following substances were prepared in similar way:

SZTP-15	$M\bar{W}$ = 11,600±200	$[\alpha]_D^{20}$ = -31.9°
SZTP-16	$M\bar{W}$ = 13,400±200	$[\alpha]_D^{20}$ = +32.6°
SZTP-17	$M\bar{W}$ = 14,500±200	$[\alpha]_D^{20}$ = +32.1°

Example 2

Poly-ε-L-lysine hydrochloride

A solution of 1 g of the polymer prepared as described in Example 1 in 40 ml of 2M NaCl solution was dialyzed against 2M NaCl solution, followed by water. After lyophilization 0.85 g of polymer was obtained.

Example 3

Poly-ε-L-lysine (free base)

An ion-exchange column (40 ml) was prepared from Dowex 1-OH anion-exchanger resin. The solution of 1 g of the polymer, prepared as described in Example 1, in 20 ml of water was chromatographed at a flow rate of 1 cm^3/min. The fractions were analyzed, collected and lyophilized. 0.65 g of polymer was isolated.

Example 4

Poly-ε-D-lysine hydrobromide

Starting from 10 g of N^{α}-benzyloxycarbonyl-N^{ε}-tert.-butyloxycarbonyl-D-lysine-p-nitrophenylester one proceeds as described in Example 1. In this way 1.3 g of polymer were obtained.

MW = 8900; $[\alpha]_D^{25}$ = -47.08° (c = 0.92; water).

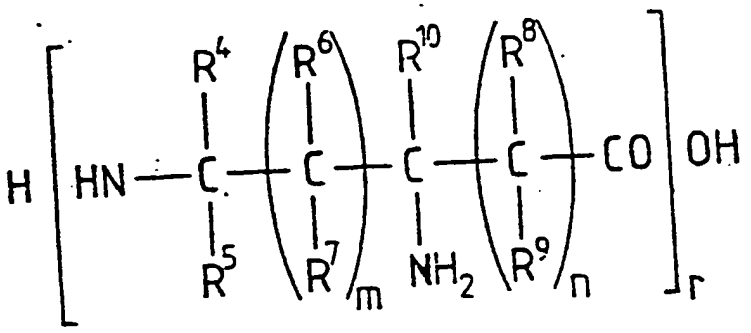
Example 5

Poly-δ-L-ornithine

According to the method described in Example 1, starting from 10 g N^{α}-benzyloxycarbonyl-(N^{δ}-tert.-butyloxycarbonil)-L-ornithine-p-nitrophenylester, 7.9 g of N^{α}-benzyloxycarbonyl-L-ornithine-p-nitrophenylester-hydrochloride were obtained. M.p.: 87-90 °C. This salt was coupled in the way described in step 1b) with N^{α}-benzyloxycarbonyl-(N^{δ}-tert.-butyloxycarbonyl)-L-ornithine into N^{α}-Z-N^{δ}(N'^{α}-Z-N'^{δ}-tert.-butyloxycarbonyl-L-ornithyl)-L-ornithine-p-nitrophenylester dipeptide (12.1 g); m.p.: 133-136 °C. Repeating step 1a), N^{α}-Z-N^{δ}-(N'^{α}-Z-L-ornithyl)-L-ornithine-p-nitrophenylester hydrochloride was prepared from the dipeptide (11.99 g; m.p.: 115-119 °C), from which N^{δ}-BOC-tri(N^{α}-Z-L-ornithine)-p-nitrophenylester (14.8 g. m.p.: 150-153 °C) was obtained by coupling according to the method of step 1b). The tri(N^{α}-Z-L-ornithine)-p-nitrophenylester salt was prepared according to the method of step 1e), m.p.: 115-130 °C. 22 g of this product were polycondensated as in step 1f), resulting in 15.4 g of δ-poly-N^{α}-benzyloxycarbonyl-L-ornithine (67.6%). From this product 13.1 g (93%) of poly-δ-l-ornithine-hydrobromide were isolated by the method of step 1g).

Claims

1. Compounds having the general formula (I)



(I)

- wherein

R^4, R^5, R^6, R^7 and R^{10} are, independently from each other, hydrogen atoms or C_{1-4} alkyl groups,

R^8 and R^9 stand for hydrogen atom each,

r is an integer from 10 to 400,

m is 0, 1, 2 or 3 and

n is 0 -

their salts, racemates and optical isomers.

2. Compounds having the general formula (I) - wherein R^4, R^5, R^6, R^7 and R^{10} are hydrogen atom each, r has the value from 10 to 400, m is 0, 1, 2 or 3 and n is 0 - and their salts, racemates and optical isomers.

3. Polyisolysine with an average molecular weight of 5500 to 25,600.

4. Polyisoornithine with an average molecular weight

of 4400 to 13,500.

5. Pharmaceutical preparations with antitumour and antiviral effect, comprising as active agent a compound of general formula (I), wherein R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} stand for hydrogen, r is an integer from 40 to 200, m is 0, 1, 2 or 3, n is 0 and the amino groups have L-configuration, in admixture with carriers and/or additives commonly used in the pharmaceutical industry.

6. Pharmaceutical preparations as claimed in claim 5, in which the active agent is a polyisolysine with an average MW of 5500 to 22,500.

7. A process for preparing polyisodiaminocarboxylic acids having the general formula (I)

- wherein

R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are the same or different and may stand for hydrogen atom or C_{1-4} alkyl groups,

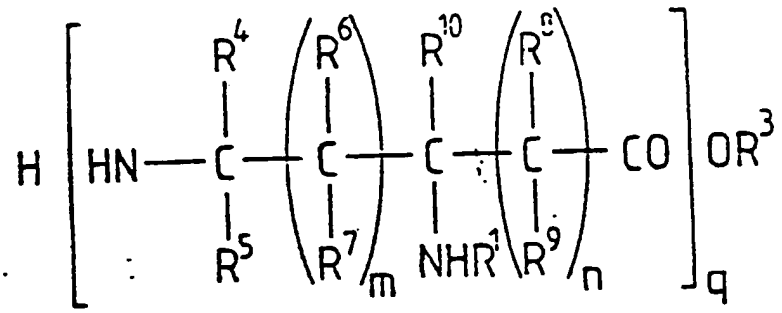
r is an integer from 10 to 400,

m is an integer from 0 to 10,

and

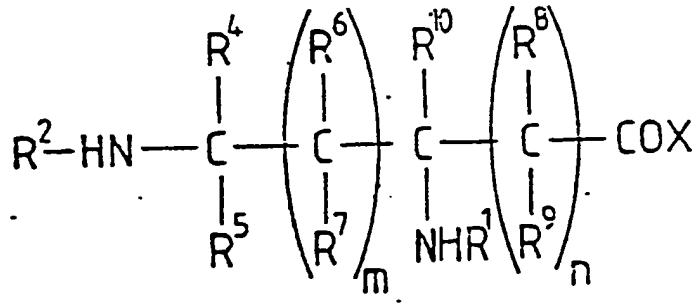
n is an integer from 0 to 10 -

their salts, racemates and optical isomers, by coupling the corresponding monomers and polyconensation of the oligomers obtained in this way, characterized in that a monomer or oligomer having the general formula (II),



(II)

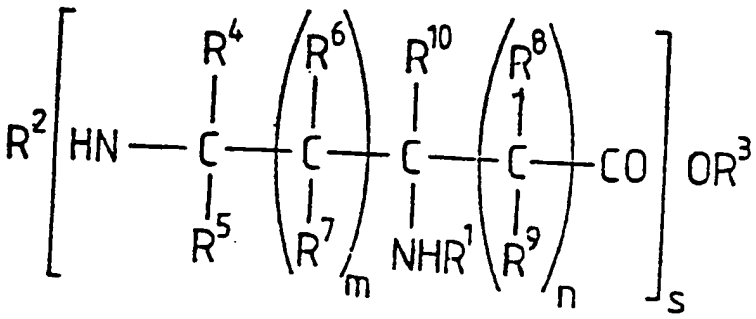
- wherein q is an integer from 1 to 9, R^1 is an amino protecting group and in oligomers all R^1 are the same, R^3 is an active ester protecting group for carboxyl groups suitable for protection and simultaneous activation, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, m and n are as stated above and in case of oligomers the substituents may be the same or different - is coupled with a monomer having the general formula (III)



(III)

- wherein R^2 is an amino protecting group different from R^1, which can selectively be removed from beside the R^1 group, X is a carboxyl-activating group with the proviso

that the -COX group is more reactive than the $COOR^3$ group, R^1, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, m and n are as stated above - and from the obtained compound of general formula (IV)



(IV)

- wherein the substituents are same as above and s is an integer from 2 to 10 - the R^2 protecting group is removed, then, if $s \leq 9$, the obtained compound is reacted with a monomer of the general formula (III) or it is polycondensated in a known way and, if desired, when the obtained compound having the general formula (I) is in free base form, it is transformed to a salt, or if it is a salt, it is transformed to a base or to another salt.

8. A process as claimed in claim 7 for producing polyisodiamino carboxylic acids of general formula (I) - wherein the value of R^4, R^5, R^6, R^7, R^{10} and r is as stated in claim 1, m is 3 and n is 0 - and their salts, characterized in that a monomer or oligomer having the general formula (II) - wherein R^1, R^3, R^4, R^5, R^6, R^7,

R^{10} and q are as stated in claim 1, and m and n are as stated above - is reacted with a monomer having the general formula (III) - wherein the meaning of R^1, R^2, R^4, R^5, R^6, R^7, R^{10} and X is as stated in claim 1, and m and n are as stated above - and from the obtained compound having the general formula (IV) - in which the substituents are as stated above and the value of s is as stated in claim 1 - the R^2 protecting group is removed and, if desired, when $s \leq 9$, the obtained product is reacted with a monomer of general formula (III) or it is polycondensated in a known way.

9. A process as claimed in claim 7 for preparing polyisodiamino carboxylic acids having the general formula (I) - wherein the meaning of R^1, R^3, R^4, R^5, R^6, R^7, R^{10} and q is as stated in claim 1, and m and n are as stated above - and their salts, in which a monomer or an oligomer of general formula (II) - wherein R^1, R^3, R^4, R^5, R^6, R^7, R^{10} and q have the same menaing as stated in claim 1, and m and n have the above meaning - is reacted with a monomer having the general formula (III) - wherein R^1, R^2, R^4, R^5, R^6, R^7, R^{10} and X are as stated in claim 1, and m and n are as stated above -, then from the obtained compound having the general formula (IV) - in which the substituents are as stated above and s is as stated in claim 1 - the R^2 protecting group is removed and, if desired, the obtained product is reacted, when $s \leq 9$, with a compound having the general formula (III) or it is polycondensed in a known way.

10. A process as claimed in claim 7 for preparing polyiso-L-lysines and their salts, characterized in that N^{α}-Z-L-lysine-p-nitrophenylester hydrochloride and N^{α}-Z-N^{ϵ}-BOC-L-lysine, transformed into a mixed anhydride with isobutyl-chloroformate, are used as starting material.

11. A process as claimed in claim 7 for preparing polyiso-L-ornithines and their salts, characterized in that N^{α}-Z-L-ornithine-p-nitrophenylester hydrochloride and N^{α}-Z-N^{β}-BOC-L-ornithine, transformed into a mixed anhydride with isobutylchloroformate, are used as starting materials.

12. A process as claimed in claim 7 for preparing polyiso-D-lysines and their salts, characterized in that N^{α}-Z-D-lysine-p-nitrophenylester hydrochloride and N^{α}-Z-N^{ϵ}-BOC-D-lysine, transformed into a mixed anhydride with isobutylchloroformate, are used as starting materials.

13. A process as claimed in claim 7 for preparing polyiso-D-ornithines and their salts, characterized in that N^{α}-Z-D-ornithine-p-nitrophenylester hydrochloride and N^{α}-Z-N^{β}-BOC-D-ornithine, transformed into a mixed anhydride with isobutylchloroformate, are used as starting materials.

14. Compounds of the general formula (I) where R_4, R_5, R_6, R_7, R_8, R_9, R_{10}, r, m and n are as defined in claim 7 (including salts thereof), when made by the process of any one of claims 7 to 13.